PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
22446PCT AJF:MM		
International Application No.	International Filing Date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/AU2003/000731	13 June 2003	14 June 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] G01N 33/48

Applicant

WOMEN'S AND CHILDREN'S HOSPITAL *et al.*

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
8 January 2004	26 May 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **NORMAN BLOM** Telephone No. (02) 6283 2238

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000731

I. Basis of the report

1. With regard to the **elements** of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-47,** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the claims, pages **50, 52, 54-56, 58-59,** as originally filed,
  page **60,** as amended (together with any statement) under Article 19,
  (Note; page 60 corresponds to and replaces original page 48 of the claims)
  pages , filed with the demand,
  pages **49, 51, 53, 57,** received on **1 April 2004** with the letter of **1 April 2004**
- [X] the drawings, pages **1/26 to 26/26,** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [ ] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**1-61**	**YES**
	Claims	**none**	**NO**
Inventive step (IS)	Claims	**1-61**	**YES**
	Claims	**none**	**NO**
Industrial applicability (IA)	Claims	**1-61**	**YES**
	Claims	**none**	**NO**

2. Citations and explanations (Rule 70.7)

Novelty (N) and Inventive Step (IS): Claims 1-61

The claims are considered to be novel and inventive in the light of any one or more of the documents cited in the ISR for the reason as outlined below.

Molecular Genetics and Metabolism, (11 March 2003), 78 (2), 193-204 discloses virtually the entire substance of the present invention but was published after the priority date of the priority document PS 2930 (see the present invention page 9 paragraph [0019]).

Biochem. J (1985), 229, 579-586 discloses that elevated levels of the sulfated N-acetylhexosamines GlcNAc6S, GalNAc6S, GalNAc4S and GalNAc4,6diS have been found in the urine of persons suffering from mucopolysaccharidosis type IIID, type IVA and type VI disease. These sulfated N-acetylhexosamines were isolated and identified by dialysis, ion exchange chromatography, paper chromatography and electrophoresis and are not derivatised or detected/quantified by mass spectrometry.

Analytical Biochemistry (1997), 248, 63-75 discloses the characterisation and quantitation of monosaccharides, maltooligosaccharides and oligosaccharides enzymatically released from asparagine-linked sites in ribonuclease B and fetuin. This characterisation and quantitation is accomplished by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) following derivatisation using 1-phenyl-3-methyl-5-pyrazolone. This document is primarily concerned with the characterisation of protein bound oligosaccharides and is silent with regard to a method for diagnosing mucopolysaccharidoses disease.

Analytical Biochemistry, (1982), 119, 120-127 discloses an improved screening test for mucopolysaccharidoses by means of **high-resolution electrophoresis** of urinary glucosaminoglycans. The GAGs, which are isolated from urine by precipitation, are not derivatised and/or purified by affinity chromatography or detected by mass spectrometry.

Clinical Chemistry (2001), 47 (11), 1937-1938 discloses the use of tandem mass spectrometry to screen newborn for inherited amino acid, organic acid and fatty acid metabolic disorders. Specifically disclosed is the disorder phenylketonuria. Analytes measured include acylcarnitines that are indicators of numerous inherited fatty acid oxidation and organic acid disorders. This document is silent with regard to the use of mass spectrometry to screen for mucopolysaccharidoses disease or the derivatisation of oligosaccharides.

Molecular Genetics and Metabolism (1998), 65, 282-290 discloses the use of a combination of anion-exchange chromatography and gradient polyacrylamide gel electrophoresis to purify and characterise urinary glucosaminoglycans from various mucopolysaccharidoses. This method, which could form the basis of a diagnostic test for MPS types and subtypes (see page 287 column 1) is based on the banding patterns produced on gradient PAGE and does not rely on determination of the target quantity of a target MPS biomarker and a reference quantity of a reference MPS biomarker using mass spectrometry. (continued)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V. 2. Citations and explanations

WO 2001/094941 discloses that the glucose tetramer (Glc1-6Glc1-4Glc1-4Glc) is a biochemical marker for POMPE disease (a lysosomal storage disorder (in particular a **glycogen storage disease** type II)). This document outlines determination of this glucose tetramer by the derivatisation with 1-phenyl-3-methyl-5-pyrazolone followed by electrospray ionization tandem mass spectrometry. POMPE disease is not a mucopolysaccharidoses and the glucose tetramer is not a glucosaminoglycan oligosaccharide cleavage product (which are used a markers for mucopolysaccharidoses in the present invention).

Clinical Chemistry (2002), 48 (1), 131-139 discloses a method of screening subjects for lysosomal storage disease (in particular **glycogen storage disease**) using the glucose tetramer α-D-Glc(1-6)-α-D-Glc(1-4)-α-D-Glc(1-4)-D-Glc. This method does not detect/quantify the tetrasaccharide by mass spectrometry and does not involve derivatization of the oligosaccharide marker.

Acta Neuropathol (2000), 100, 409-414 discloses the post-mortem diagnosis of **G_{M1}-gangliosidosis** in a dog by the detection of elevated levels of G_{M1}-ganglioside by electrospray ionization tandem mass spectrometry of glycolipids extracted from formalin-fixed brain tissue. G_{M1}-gangliosidosis is not a mucopolysaccharidoses and the glycolipid markers are distinct form the oligosaccharide GAG cleavage products used as markers for mucopolysaccharidoses in the present invention.

Anal. Chem. (2001), 73, 1651-1657 discloses a method for the quantitative determination of enzyme activities of four heparin-modifying enzymes in human cell lysates by contacting a substrate-linker-biotin conjugate (and as internal standard a product-isotope labelled linker-biotin conjugate) with a crude lysate from skin fibroblasts. The product-linker-biotin conjugate produced is isolated by affinity capture (biotin-avidin/streptavidin binding) and analysed by ESI-MS. The substrate conjugate, referred to above, is quite distinct from an oligosaccharide biochemical marker of mucopolysaccharidoses found in a biological sample from an individual.

The Journal of Investigative Dermatology (1982), 79 Supplement 1, 38s-48s outlines the biochemistry and clinical presentations of mucopolysaccharidoses, including clinical classifications of the mucopolysaccharidoses and the enzymes involved in glucosaminoglycan catabolism. This document indicates that the critical laboratory finding in MPS is an elevated GAG level in the urine where the class of GAG excreted may be used to differentiate between the MPS types. This document does not suggest derivatisation of GAGs found in urine or that they may be detected and quantified using mass spectrometry.

Glycoconjugate Journal, (1998), 15, 737-747 discloses a method of desalting oligosaccharides released from glycoproteins using graphitized carbon as a solid phase adsorbent. This method is said to be advantageous for purification of oligosaccharides prior to electrospray mass spectrometry. This document is silent with regard to a method of diagnosing the clinical status of a mucopolysaccharidoses disease.

RU 2196988 (the English abstract of this patent application) discloses a method for diagnosis of mucopolysaccharidoses by treatment of separate types of glucosaminoglycans with specific enzymes (chondroitinases AC and ABC) followed by single dimensional electrophoresis and does not appear to involve derivatisation and/or mass spectrometry.

J. Amer. Soc. Mass Spectrom. (2000), 11, 916-920 discloses a method using electrospray ionization mass spectrometry to identify and quantify the sulfated disaccharide building blocks of glucosaminoglycans that are released following enzymatic digestion. This document indicates that GAGs play key roles in many diseases such as mitogenesis, angiogenesis and inflammation. This document is silent with regard to mucopolysaccharidoses disease and does not teach the derivatisation of the disaccharides.

WO 2003/048784 discloses an internal standard for use in mass spectrometry to determine the concentration of a test **sphingolipid**. The internal standard comprising the same oligosaccharyl chain and long chain base sphingosine as the test compound. This test standard is useful in the diagnosis of **glycosphingolipidises** which constitute a large group of lysosomal storage diseases.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for diagnosing a pre-clinical status, or a clinical status of a mucopolysaccharidoses ("MPS") disease in a target animal comprising:

(a) determining a target quantity of a target MPS biomarker from a target biological sample taken from the target animal; and

(b) comparing the target quantity to a reference quantity of a reference MPS biomarker;

wherein,

the target MPS biomarker is the same or equivalent to the reference MPS biomarker, and each of the target MPS biomarker and the reference MPS biomarker is an oligosaccharide;

the reference quantity is determined from a reference animal, or group of reference animals, having a known MPS clinical status; and

a deviation of the target quantity of the target MPS biomarker from the reference quantity of the reference MPS biomarker is a pre-clinical or clinical indication of the MPS disease, an indication of a progression of the MPS disease, or an indication of a regression of the MPS disease.

2. The method of claim 1, wherein the target biological sample or reference biological sample is selected from a cellular extract, blood, plasma, or urine.

3. The method of claim 1, further comprising derivatizing the target MPS biomarker and the reference MPS biomarker with a derivatizing agent prior to determining the quantity of the target MPS biomarker or the quantity of the reference MPS biomarker .

4. The method of claim 3, wherein the derivatizing agent comprises 1- phenyl-3-methyl-5-pyrazolone ("PMP").

5. The method of claim 1, wherein the oligosaccharide comprises a sulfated molecule having a sugar length ranging from 1 to 12 residues.

6. The method of claim 1, wherein the oligosaccharide identified from the target biological sample comprises a cleavage product of a glycosaminoglycan ("GAG").

7. The method of claim 6, wherein the GAG is heparan sulfate, dermatan sulfate, keratan sulfate, or chondroitin sulfate.

8. The method of claim 1, wherein the oligosaccharide is a dermatan sulfate fragment that comprises: IdoA-(GalNAc-(UA-GalNAc)$_n$)(S)$_m$, wherein, n=0-5, m=0-11; IdoA-(GalNAc-UA)$_n$(S)$_m$, wherein, n=1-6, m=0-12 ; IdoA2S-(GalNAc-(UA-GalNAc)$_n$)(S)$_m$, wherein, n=0-5, m=0-11; IdoA2S-(GalNAc-UA)$_n$(S)$_m$, wherein, n=1-6, m=0-12; GalNAc4S-(UA-(GalNAc-UA)$_n$)(S)$_m$, wherein, n=0-5, m=0-12; GalNAc4S-(UA-GalNAc)$_n$(S)$_m$, wherein, n=0-6, m=0-13; GlcA-GalNAc-(UA-GalNAc)$_n$)(S)$_m$, wherein, n=0-5, m=0-11; or GlcA-(GalNAc-UA)$_n$(S)$_m$, wherein, n=0-6, m=0-12;

wherein,

IdoA = iduronic acid; GlcA = glucuronic acid; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; GlcN = glucosamine; UA = uronic acid; S = sulfate; and Gal = galactose.

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12. The method of claim 1, wherein the target quantity and the reference quantity are determined by a mass spectrometric analysis.

13. The method of claim 1, wherein the target quantity and the reference quantity are determined by a chromatographic assay, an immunoassay, liquid chromatography, anion exchange chromatography, size exclusion chromatography, or combination thereof.

14. The method of claim 1, wherein the target quantity and the reference quantity are normalized to creatinine or another oligosaccharide.

15. The method of claim 1, wherein the target animal has received a MPS therapy.

16. The method of claim 15, wherein the MPS therapy comprises a bone marrow transplant ("BMT") or a MPS enzyme replacement therapy.

17. The method of claim 1, further comprising treating the target animal with a MPS therapy, wherein the MPS therapy is based on the deviation of the target quantity as compared to the reference quantity.

18. The method of claim 17, wherein the MPS therapy comprises a bone marrow transplant ("BMT") or a MPS enzyme replacement therapy.

19. The method of claim 1, wherein the target biological sample and the reference biological sample contain an internal standard.

20. The method of claim 19, wherein the internal standard comprises a deuterated N-acetylglucosamine-6-sulfate ("GlcNAc6S(d3)").

the target MPS biomarker was obtained from a biological sample of a target animal having the MPS biomarker contained therein;

the target MPS biomarker is the same or equivalent to the reference MPS biomarker, and each of the target MPS biomarker and the reference MPS biomarker is an oligosaccharide;

the reference quantity is determined in a reference animal, or group of reference animals having a known MPS clinical status; and

a deviation in the quantity of the eluted target MPS biomarker when compared to the reference quantity is a pre-clinical or clinical indication of the MPS disease, a progression of the MPS disease, or a regression of the MPS disease.

28. The method of claim 27, wherein the target biological sample or reference biological sample is selected from a cellular extract, blood, plasma, or urine.

29. The method of claim 27, further comprising lyophilizing the target biological sample prior to derivatizing the target MPS biomarker.

30. The method of claim 27, wherein the derivatizing agent comprises 1- phenyl-3-methyl-5-pyrazolone ("PMP").

31. The method of claim 27, wherein the oligosaccharide comprises a sulfated molecule having a sugar length ranging from 1 to 12 residues.

32. The method of claim 27, wherein the oligosaccharide identified from the target biological sample comprises a cleavage product of a glycosaminoglycan ("GAG").

33. The method of claim 32, wherein the GAG is heparan sulfate, dermatan sulfate, keratan sulfate, or chondroitin sulfate.

(c) an internal standard;

(d) a solid phase extraction column;

(e) a solid phase extraction column wash solution; and

(f) an oligosaccharide elution solution.

53. The kit of claim 48, wherein the oligosaccharide derivatization agent is a solution comprising: 1-phenyl-3methyl-5-pyazolone ("PMP").

54. The kit of claim 48, wherein the acid solution is a vial comprising: formic acid

55. The kit of claim 48, wherein the internal standard comprises: a deuterated N-acetylglucosamine-6-sulfate ("GlcNAc6S(d3)").

56. The method of claim 48, wherein the internal standard comprises a non-physiological oligosaccharide that is similar to the oligosaccharide being investigated.

57. The method of claim 52, wherein the non-physiological oligosaccharide is derived from a chondroitinase digestion of chondroitin sulfate having an unsaturated uronic acid at the non-reducing end.

58. The method of claim 48, wherein the solid phase extraction column comprises a C18 reverse phase column.

59. The method of claim 48, wherein the solid phase extraction column wash solution comprises: $CHCl_3$.

60. The method of claim 48, wherein the oligosaccharide elution solution comprises: CH_3CN and formic acid.

61. A method for diagnosing a pre-clinical status, or a clinical status, of a mucopolysaccharidoses ("MPS") disease in a target animal comprising: